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Cuticular wax from flax processing waste with hexane and super critical carbon dioxide extractions

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Abstract

The waste material produced when flax fiber is processed has potentially valuable co-products. The dust was extracted with CO_2 under super critical fluid extraction (SCFE) conditions or hexane to isolate the cuticular waxes that contain policosanols. Policosanol is a general term for long chain alcohols and have been shown to improve blood chemistry. SCFE yielded 7.4% wax compared with 4.0% with hexane. There were slight but statistical differences in extract compositions for C-20, C-22, and C-26 alcohols and larger significant differences for C-16, C-18, and C-20 fatty acids depending upon the extraction methods used. Recovery of wax from the waste produced from flax processing can provide an attractive value added product with positive health benefits.
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1. Introduction

Long chain alcohols, policosanols, improve blood chemistry by lowering low-density lipoproteins (LDL) and increasing high-density lipoproteins (HDL) as well as lowering total cholesterol. They are also anti-aggregatory without the side effects often reported with some currently used drugs (Taylor et al., 2003; Varady et al., 2003). Policosanols are present in the wax coating of the cuticle present on most plants that acts to control respiration and pathogen entry. A commercial product containing purified policosanols is derived from hydrolytic cleavage of purified sugar cane wax (Gouni-Berthold and Berthold, 2002). Octacosanol is thought to be one of the major policosanols responsible for this improvement in blood chemistry (Taylor et al., 2003). The benefits of similar phytochemicals in sorghum have been extensively reviewed (Awika and Rooney, 2004). We have shown that the cuticle of the flax plant is rich in these compounds (Morrison and Akin, 2001), and Gutierrez and Del Rio (2003) have provided an excellent detailed analysis of the lipids from flax fiber and how they are affected during pulping. When retted flax is processed for fiber, a large amount of "dust" is produced in which the cuticle is concentrated. This dust is considered waste and must be disposed of in some way, such as a landfill or burning.

Utilization of the waste material could provide a valuable co-product that would help the flax industry

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by providing a value added product. Extraction of this dust yields a wax rich in policosanols that is suitable as a nutraceutical, thus adding increased value to flax fiber produced. This report will present a comparison of the results of solvent extraction with hexane and super-critical fluid extraction with carbon dioxide using dust produced in the waste stream during processing of flax. Yields and composition of the extracts will be presented.

2. Experimental

2.1. Samples

Flax "dust" was obtained from dew-retted flax from a diverse set of commercial bales that had been processed through a Unified Line (Czech Flax Machinery, Merin, CR) line, and further refined and cottonized. The waste from these processing steps constituted the flax "dust" and was collected in the bag house (the collection site for fines and dust removed by exhaust fans along the processing line). This waste material was made up of fiber and plant cuticle that had been stripped from the fiber. Flax dust was then obtained by shaking a bag containing the trash and removing the fines from the bottom of the bag.

2.2. Solvent extraction

Approximately 2 g flax dust was extracted in a 2050 Soxtec autoextractor (Foss Tecator, Höganäs, Sweden) with hexane for 6 h. After removal of solvent and drying in a vacuum oven using a tared flask, the weight of the residue was recorded and the extract analyzed by gas chromatography. A rapid extraction was performed in which 1 g was placed in 100 ml boiling hexane for 30 s, filtered, and the solvent evaporated. A second extraction of this material yielded no additional extract. Extractions were performed in triplicate.

2.3. Super critical fluid extraction (SCFE)

Extractions were performed with an Isco Model SFX 3560 Extractor (Teledyne Isco Inc., Lincoln, NE) at 60 °C and 5.52×10^7 Pa. Samples (1 g) of solid material were placed into stainless extraction vials and extracted with 99.99% purity carbon dioxide (Airgas Inc., Radnor, PA) followed by sequential extractions with mixtures of 1%, 5%, or 10% (v/v) absolute ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY) in CO_2 at a flow rate of 1 ml/min for 60 min. Extracts were collected at 25 °C and fractions collected after each step in the extraction sequence. Extractions were performed in triplicate.

2.4. Gas chromatographic analysis

Gas chromatographic (GC) analyses of fatty acid alcohols, fatty acids and aldehydes were conducted on a HP 6890N (Agilent Technologies, Palo Alto, CA) gas chromatograph fitted with a 30 m, 0.32 mm i.d., HP-5 capillary column (0.25 μm film thickness) (Agilent Technologies, Palo Alto, CA). The GC injector was set at 275 °C and the FID detector at 300 °C. Oven temperature was programmed with a 170 °C, 5 min hold followed by 10 °C/min to 275 °C with a 50 min hold. Heptade-canoic acid was used as an internal standard for acids, alcohols, and aldehydes. Stearyl behenate (a C-40 ester) was the internal standard for long chain esters. Stock solutions of internal standards and extracts in hexane of each of the triplicate extracts for each treatment were prepared. An aliquot was removed and the solvent evaporated prior to analysis. Samples were first treated with 5 ml 14% BF_3/methanol to form the methyl ester of the free acids. After extraction with 2×1 ml hexane, the solvent was evaporated and pyridine and (BSTFA) N,O-bis(trimethylsilyl) triflouroacetamide was added to form the silyl ethers of the remaining alcohols. This procedure results in base line separation of fatty acid methyl esters, silylated alcohols, and free aldehydes. To ensure that these conditions did not hydrolyze waxy esters, stearyl stearate was treated under these conditions. No octade-canol or stearic acid was produced.

For analysis of long chain esters, aliquots of the preceding stock solutions were treated as described and analyzed using a 10 m HP-5 column as previously described. Oven conditions were: 170 °C for 1 min, 10 °C/min to 325 °C with a 50 min hold. Waxes were analyzed as their long chain esters.

2.5. GC mass spectrometry (MS) analysis

GC–MS analysis was conducted on a Thermo Quest Polaris Q (Thermo Electron Corp., Madison, WI) at 70 eV using the same chromatographic columns and conditions as described. Compounds were identified by comparing their spectra with the NIST library and those of authentic standards.

2.6. Thin layer chromatography

Thin layer chromatography was conducted using 20 cm × 20 cm silica gel G (0.25 mm) (Sigma–Aldrich, St. Louis, MO) plates. Approximately 0.75 mg of hexane extract was dissolved in hexane, streaked, and eluted with hexane/diethyl ether/acetic acid (90/10/1 (v/v/v)). A 1 cm section of the side of the plates was sprayed

with chromic acid–sulfuric acid reagent followed by heating at $120°$ C to visualize the plate. After visualization, the untreated portion of the each band was marked and removed from the plate. Under these conditions, the Rf values were as follows: fatty alcohols, 0.06; fatty acids, 0.11; aldehydes, 0.53; wax esters, 0.79. Each band was extracted with $2\times$ 10 ml portions of boiling hexane. Samples were analyzed neat and as their silyl ethers as described. The compounds were identified by comparing their GC retention times and their mass spectra with authentic compounds or reference spectra. The isolation of each class of compounds provided easier identification of those components that were co-eluted on the gas chromatograph when only BSTFA was added.

3. Result and discussion

The results of extractions performed on flax dust using hexane and (SCFE) with CO_2 are shown in Table 1. Extraction with CO_2 is the most efficient giving a yield of 7.4% wax. An exhaustive extraction using the Soxtec autoextractor with hexane yielded about 4% wax. Supercritical CO_2 is known to be very effective for the extraction of lipid compounds and has been successfully applied to oilseeds and similar materials (Bully et al., 1984). A rapid extraction with hexane that involved simply immersing the dust in boiling hexane for 30 s yielded about 4.5% extract. Yield will vary depending on the amount of fiber and other trash that is carried over in the trash stream. A bulk sample of this trash prior to isolation of the fine dust yielded only about 2% wax by hexane extraction. Repeated extractions with hexane did not improve yields. Separation of the wax using thin layer chromatography separated the components of the wax and further identification by GLC and GCMS. Quantitation of the hexane and CO_2 extracts yielded about 780 mg g^{-1} of the major compounds with another 200 to 220 mg g^{-1} that was made up of between 20 and 30 compounds.

Gutierrez and Del Rio (2003) identified several sterols and triterpenes. Our isolates contained only trace amounts of these compounds. The CO_2 extract and a CO_2/ethanol SCFE yielded slightly more than the hexane extract. A sequential SCFE was conducted with CO_2, and 1, 5, and 10% ethanol in CO_2. Of the total amount extracted, 93–94% was extracted with the initial CO_2 extraction. The additional material removed with the addition of ethanol consisted of residual fatty acids, alcohols, and trace amounts of sterols. The use of a co-solvent such as ethanol with CO_2 can facilitate the extraction of polar compounds such as phospholipids. This procedure provides a technique to obtain lipid fractions of varying

polarity by adjusting the proportion of co-solvent. Such an approach was used effectively to separate neutral and polar lipids from *Hibiscus* seeds (Holser et al., 2004).

Difference in composition of the waxes was present between the two extraction methods (Table 1). Long chain alcohols, policosanols, represented the largest group of compounds with about 350–380 mg g^{-1} wax. The most abundant as expected was octacosanol (C-28) averaging about 60% of the alcohols with triacontanol (C-30) the next most abundant averaging 30% and the yield being essentially equal for each extract. Of

Table 1
Composition of the long chain alcohols, acids, wax esters, and aldehydes removed from flax dust with hexane and CO_2

Compounds	Hexane (%)	Carbon dioxide (%)
Yield	$4.0 \pm 0.1\%$[*]	$7.4 \pm 0.5\%$[*]
Alcohols (total mg g^{-1})	(347 ± 70)	(384 ± 54)
% of total alcohols		
C-18	0.61 ± 0.23	0.33 ± 0.02
C-20	0.22 ± 0.01[*]	0.28 ± 0.01[*]
C-22	0.14 ± 0.01[*]	0.19 ± 0.02[*]
C-24	0.53 ± 0.05	0.58 ± 0.02
C-26	9.6 ± 0.2[*]	10.2 ± 0.1[*]
C-28	60.5 ± 4	59.1 ± 0.8
C-30	30.1 ± 0.6	29.8 ± 0.6
Fatty acids (total mg g^{-1})	(124 ± 29)	(135 ± 25)
% of total fatty acids		
C-16	11.9 ± 0.6[*]	17.8 ± 0.2[*]
C-18	17.4 ± 0.4[*]	25.0 ± 4.6[*]
C-20	4.4 ± 0.4[*]	5.9 ± 0.7[*]
C-22	5.5 ± 0.9	6.6 ± 0.5
C-24	5.3 ± 3	8.0 ± 0.5
C-26	8.3 ± 1	7.5 ± 1
C-28	18.2 ± 4	17.0 ± 1
C-30	18.7 ± 4	18.1 ± 1
C-32	Trace	Trace
Aldehydes (total mg g^{-1})	(23.4 ± 4)	(34.1 ± 10)
% of total aldehydes		
C-24	Trace	Trace
C-26	7.2 ± 2	8.2 ± 1
C-28	41.7 ± 5	38.7 ± 0.4
C-30	50.8 ± 4	52.9 ± 1
Wax esters (total mg g^{-1})	(285 ± 64)	(226 ± 30)
% of total wax esters		
C-48	1.55 ± 0.4	1.9 ± 0.2
C-50	14.7 ± 1	18.9 ± 3
C-52	46.8 ± 4	44.1 ± 2
C-54	24.6 ± 3	22.1 ± 4
C-56	6.0 ± 0.5	5.1 ± 0.7
C-58	3.7 ± 0.3	3.0 ± 0.3
C-60	2.8 ± 0.4	2.2 ± 0.4

Another 200–300 mg g^{-1} constituted another 20–25 minor identified and unidentified products in each extract.
[*] $P < 0.05$.

the minor alcohols, octadecanol (C-18) was found in greater amounts in the hexane extract, but not significantly greater. Although there are significant differences in the amounts of eicosanol (C-20), docosanol (C-22), and hexacosanol (C-26) found in the two extracts with the CO_2 extract containing the greater amount, their percentages are relatively small.

The three fatty acids, palmitic (C-16), stearic (C-18), and isocosanic (C-20 were significantly higher in the CO_2 extract and appear to be more efficiently extracted with this solvent. Octacosanoic (C-28) and triacontanoic (C-30) acids were the most abundant in the hexane extract, but were not significantly higher than in the CO_2 extract.

The aldehydes, which made up the smallest group, were made up of the carbon chain C-26, C-28, and C-30 and have almost equivalent composition between the two extraction methods.

The wax esters were the second largest group where the amount of each chain length agreed well with that of Gutierrez and Del Rio (2003). Our analysis of the individual ester was not as complete, but examination of the fatty acids and alcohols of the individual esters reported by Gutierrez and Del Rio (2003) reveals that hydrolysis of these waxes would not significantly increase the amount of octacosanol.

4. Conclusions

Two methods for the isolation of flax wax containing a promising nutraceutical have been investigated. SCFE with carbon dioxide produces a high yield without the use of a flammable solvent. Compositions of extracts with CO_2 and organic solvent were almost identical and agreed with an earlier study (Gutierrez and Del Rio, 2003). Although there were some differences in the concentrations of palmitic and stearic acids between extraction methods, the concentration of the policosanol thought to be the most important nutraceutical, octacosanol, exhibited no difference.

The significantly higher recovery of extract obtained with CO_2 extraction suggests further consideration for the possible large-scale isolation of the wax from the flax dust. The use of carbon dioxide requires more sophisticated process equipment, e.g. pressure vessels and instrumentation, than typically needed for organic solvent extraction. However, other factors must be considered. Carbon dioxide is renewable. It is obtained as an inexpensive, high purity by-product from fermentation processes inexpensively and at very high purity. Additionally, it is not flammable and non-toxic. In a typical process, the carbon dioxide is recycled and the extract is simply recovered by reducing the system pressure. Extraction with carbon dioxide is especially applicable to recover thermally labile natural products in high yield.

The most attractive aspect of this work is the use of a waste material produced during flax processing that can yield a potentially valuable nutraceutical shown to improve blood chemistry.

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